REMARKS

Upon entry of the present amendments, claims 1, 3-8, 11-14, and 20-31 are pending.

Applicants have canceled claims 2, 9-10, and 15-19 without prejudice or disclaimer. Applicants have added new claims 21-31. Support for the recitation of --mammalian non-plasmocyte cell-- is found in the specification on pg. 4, para. 3. Support for the recitation of --a single antibody heavy chain-- and --a single antibody light chain-- is found in the specification at pg. 12, lines 9 and 20. Support for the recitation of --two distinct nucleic acid-- in claims 23 and 25 is found in the specification at pg. 13, TABLE 1. Support for the recitation of --wherein one nucleic acid comprises a nucleotide sequence coding for an heavy chain of an antibody molecule and the other nucleic acid comprises a nucleotide sequence coding for a light chain of an antibody molecule-- in claim 25 is also found in the specification at pg. 13, TABLE 1. Support for --with a nucleic acid, wherein the nucleic acid comprises one nucleotide sequence coding for one fragment of an antibody molecule and an another nucleotide sequence coding for another fragment of an antibody molecule-- in claims 26 and 29 is found in the specification, pg. 13, lines 1-2 (pLXPXSN). Support for --IRES sequence-- is found in the specification at pg. 13, line 5. Support for --anti-human thyroglobin (anti-Tg10)-- is found in the specification at pp. 12-13. Support for --implanting a cell into a mammal-- is found in the specification at pg. 15, line 17. No new matter is added.

Applicants have amended claims 1, 3-8, 11-14, and 20 to depend from new claim 21. Applicants have also amended claims 1, 3-8, 11-14, and 20 as suggested by the Examiner (*see,* below). Support for --wherein the antibody polypeptide does not induce an immune response neutralizing the antibody polypeptide-- is found in the specification at pg. 16, lines 13-14. Support for --wherein the mammalian non-plasmocyte cell is genetically modified *in vitro* before being introduced into the host mammal-- is found in the specification at pg. 4, lines 21-24. No new matter is added.

The Examiner has objected to the specification, alleging that the specification is not clear, concise, and exact. Applicants have revised the specification as suggested by the Examiner. Applicants thank the Examiner for the suggestions. No new matter is added.

The Examiner has objected to the ABSTRACT, alleging that the Abstract uses legal terminology. Applicants have corrected the ABSTRACT as suggested by the Examiner. Applicants thank the Examiner for the suggestions. No new matter is added.

Applicants acknowledge that FR 2,706,486 was only considered with respect to the English abstract, since no translation was supplied.

35 USC § 101

The Examiner has rejected claims 17-19 under 35 U.S.C. § 101, because the claimed recitation of a allegedly results in an improper definition of a process. Applicants have canceled claims 17-19. This rejection is now moot and should be withdrawn.

35 USC § 112, second paragraph

The Examiner has rejected claims 1-20 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. The Examiner has also objected to claims 1-5, 12-16, and 20 (and dependent claims). Applicants have added new claims 21-31 and amended claims 1-20 to depend from the new claim 21. Applicants have also amended the claims as suggested by the Examiner The new claims and amendments obviate the Examiner's rejections of and objections to the previous claims.

Applicants have amended the form of the claims, so that each claim is the object of a sentence starting with "We claim."

Claim 1

New claim 21 recites a --mammalian non-plasmocyte cell—rather than a "biological material". New claims 21-31 do not recite the term "its previous culture".

Applicants have amended claim 1 to remove recitation of or provide antecedent basis for the terms "the secretion" (line 9), "the blood circulation" (line 9), and "this antibody or a fragment of it" (line 10) lacks antecedent basis.

Applicants have amended claim 1 to remove recitation of the phrases "in a form enabling in the transfer" in line 3; "in a form enabling its incorporation into the mammal's organism" in lines 5 and 6; "previous culture" in line 6; and "enabling its incorporation"; "the mammal not naturally producing antibodies" in line 4; "a previous nucleic acid sequence" in line 5; "said nucleic acid" in line 7; "by cells" in line 10; and "genetically modified" in lines 4 and 10.

Applicants have amended claims 1-4 and 20 to remove recitation of the phrase "not naturally producing antibodies".

Applicants have amended claims 1-4, 6, 12, and 20 to remove recitation of the term "gene".

Applicants have amended claims 1 and 6 (and dependent claims) to remove recitation of the phrase "and elements for expressing *in vivo* said antibody gene and the secretion in the blood circulation of a mammal of a therapeutically effective amount of this antibody" and the phrase "and elements guaranteeing the expressing in vivo of said antibody gene and the secretion in the blood circulation of a mammal of a therapeutically effective amount of this antibody"

Claim 2

Applicants have canceled claim 2.

Claim 3

Applicants have amended claims 3 and 4 to be single sentences.

Applicants have amended claim 3 to remove recitation of the terms "this sequence is a complex"; and the "molecule" in line 6.

Applicants have amended claim 3 to remove recitation of the "sequence" being "conjugated".

Claim 4

Applicants have amended claim 4 to remove recitation of the "sequence" being "a vector".

Applicants have amended claim 4 to remove recitation of the terms "permitting" and "effective transfer" in line 6.

Claim 5

Applicants have deleted the term "biological" in claim 5, line 2.

Claim 6

Applicants have amended claim 6 to remove recitation of the term "form" in line 2; the phrase "permit[ting] their incorporation into the mammal's organism"; the term "its previous culture"; and the term in line 6 "this antibody or a fragment of it".

Claim 7

Applicants have amended claims 7 and 8 to remove recitation "cells" and "mammal" in line 3 of claim 7 and in lines 3 and 4 of claim 8.

Applicants have amended claims 7 and 8 to remove recitation in line 3 of "the cells" and "come from".

Claim 8

Applicants have amended claim 8 to remove recitation of the phrase "and have undergone treatment"; the term "them" in line 4; the term "compatible" in line 5; and the term "treatment".

Claims 9 and 10

Applicants have canceled claims 9 and 10.

Claim 12

Applicants have amended claim 12 to remove recitation of the term "derivative".

Applicants have amended claim 12 to remove recitation of the term "virgin antibody" in line 2. Regarding the definition recited on p. 5, lines 7, 8, Applicants note that the claims as amended overcome the Examiner's rejection, since the heavy chain of a "natural antibody" is encoded by "a gene".

Applicants have amended claim 12, in response to the Examiner's suggestion, to recite the term --chimeric-- rather than "chimerical". Applicants thank the Examiner for this and other suggestions.

Claim 13

Applicants have amended claim 13 to remove recitation of the term, in line 3, "[said] antibody derivative".

Applicants have amended claim 13, line 4, and claim 14, lines 4 and 5, to delete the term "specific".

Claim 14

Applicants have amended claim 14 to remove recitation of the terms "said antibody fragment" in line 3; "[said] antibody derivative" in lines 3 and 4; "the virus" in line 4; and "cells" in line 5. Thus, the issue of lack antecedent basis has been addressed.

Claim 15

Applicants have canceled claim 15.

Claim 20

Applicants have amended claim 20 to remove recitation of the phrase "by cells of said mammal genetically modified by said nucleic acid sequence and not producing antibodies naturally in cells not naturally producing antibodies".

Applicants have amended claim 20 to recite that the "manufacturing process" is a method of making, and to include the steps of the process. Claim 20 recites a process for making a mammalian non-plasmocyte cell.

Accordingly, Applicants request that the rejections under 35 U.S.C. § 112, second paragraph be withdrawn.

35 USC § 112, first paragraph, enablement

The Examiner has rejected claims 1-20 under 35 U.S.C. § 112, first paragraph, as allegedly not enabled. Applicants respectfully traverse.

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. MPEP § 2164.01. The amount of experimentation that is permissible to provide enablement depends upon a number of factors, which include: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability of the art, and (8) the breadth of the claims. *In re Wands,* 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988); MPEP § 2164.01. Here, one skilled in the art could, with the guidance of the specification and the teachings in the art, make the claimed mammalian non-plasmocyte cell, genetically modified with a nucleic acid coding for an antibody molecule, without undue experimentation. Applicants show that an antibody made by cells that do not naturally produce antibodies, when implanted into a whole mammal organism, can be secreted outside the cell then diffused throughout interstitial fluids to reach the blood circulation. The graft of the *ex vivo* genetically modified cells as disclosed by the specification constitutes a good demonstration.

Nature of the invention and state of the prior art

The use of the invention involves the use of the cells of the invention. Thus, the use of the invention would not be the "gene therapy" described by French Anderson, 392 (Supp.) Nature 25 (1998), first paragraph, or Verma & Somia, 389 Nature 239 (1997), col. 3, 2nd paragraph.

By contrast, the approach disclosed by the Applicants involves the transfer of particular genes to particular cells, allowing the expression and secretion of proteins encoded by those genes. This approach is supported by results of experiments by the Applicants relevant to the present invention.

Moreover, gene therapy has developed considerably since 1998. Under this continuous development, the design and elaboration of efficient gene delivery systems now allows for more effective clinical trials. Some recent results allows those skilled in the art to believe that gene therapy is becoming an important tool in treatment of human genetic diseases (*see,* French Anderson, 288(5466) Science 627-9 (2000) (copy enclosed)). Recent clinical trials have shown that, depending in clinical protocols and experimental approaches, gene therapy is becoming the best way for therapeutic treatment of human gene failures. For example, Cavazzana-Calvo *et al.*, 288 Science 669-672 (2000) recently reported an important success concerning the gene therapy of a child immunodeficiency (copy enclosed).

Human myoblast grafts are currently being attempted by many research laboratories; progress is being made. The Applicants have themselves conducted laboratory experiments showing that muscle cells are not the only cells that can provide assuring systemic and long term delivery of an ectopic monoclonal antibody *in vivo*. Murine skin fibroblasts and reconstituted human skin are also able to produce similar effects. Moreover, intramuscular or intravenous injection of recombinant adenovirus also induces an ectopic monoclonal antibody production, which persists for several months.

The approach proposed by the Applicants should contribute to the optimization of gene therapy protocols. We should not reject future developments of any gene therapy approach only on the basis of past failures. We should not found arguments in scientific literature to stop gene therapy.

Breadth of claims

The claims as amended are not broad. The claims recite cells and methods of making these cells. The invention concerns a new antibody delivery system, based on the production and external secretion of antibodies by genetically modified cells that do not naturally produce or secrete antibodies. The breadth of the application field results from the many possibilities offered by antibody diversity, rather than the scope of the claimed subject matter.

Predictability of the art

The claims concern the genetic transformation of a cell that does not naturally produce and secrete antibodies cell into cells that produce and secrete antibodies. The antibody production and secretion are maintained for a long period of time. Because the Applicants have reduced to practice the obtaining of such genetically modified cells, as shown in the specification (pp. 12-16), the claimed antibody- secreting cells should not be considered as "unpredictable".

Guidance and working examples

The Examiner alleges the specification provides little guidance teaching one of ordinary skill in the art how to make and use the claimed invention in the treatment of any disease by *in vivo* or *ex vivo* gene therapy. Applicants respectfully disagree.

It should not be necessary today to prove that a monoclonal antibody can be used as a therapeutic agent. Such uses of monoclonal antibodies are widely disclosed in the literature for the treatment of both human and animal pathologies. It is known that the only requirement is to inject the purified monoclonal antibody into the patients (*see,* Glennie & Johnson, 21(8) Immunol Today 403-10 (2000). It is also known that one of the most important conditions for an efficient immunotherapy treatment is the delivery of an efficient amount of antibody either into the blood circulation or beside the site where the antibody must react.

The claimed invention provides a new monoclonal antibody delivery method that eliminates the undesirable effects due to invasive administration of enormous amounts of therapeutic monoclonal antibody. The method thus also reduces treatment cost. Accordingly, the unique model monoclonal antibody (anti-Tg10), whose production and secretion are easily measured, and to which any possible anti-idiotype response can be detected, is enough to validate the delivery method using the novel genetically engineered antibody secreting cells.

The Examiner alleges that the specification does not teach how to make embodiments guaranteeing the expression *in vivo* of an antibody gene and the secretion in the blood circulation of a mammal. Applicants respectfully disagree. The specification provides that in addition to the antibody gene and his promoter, the nucleic acid sequence can include a polyadenylation sequence situated downstream from the antibody gene for proper termination of RNA transcription, resulting in the secretion of the antibody gene product into the blood circulation of the mammal (specification, pg. 5, lines 14-18).

The Examiner also alleges that the specification does not disclose what the termination sequences are or how they guarantee or permit secretion in the blood circulation of a mammal. Applicants again respectfully disagree. Many expression vectors exist that allow those skilled in the art to transfer and express antibody genes. The Applicant discloses, for example, the use of a PM130 vector, which contains two leader nucleotide sequences encoding respectively for heavy chain signal peptide and for light chain signal peptide, both signal peptides being useful for the secretion of the antibody molecule outside the cell (*see,* specification, pg. 13, TABLE 1). Figure 1a represents both polynucleotide and polypeptide sequence for the heavy chain variable region of Tg10 antibody. The first 20 amino acids, from position –20 to position –1, correspond to the signal peptide sequence coding for the heavy chain signal peptide. Figure 2a represents both polynucleotide and polypeptide sequence for the light chain variable region of Tg10 antibody. The first 20 amino acids, from position –20 to position –1, correspond to the signal peptide sequence coding for the light chain signal peptide. Accordingly, the specification does provide an enabling description of leader sequences.

The Examiner alleges that the assertion of the claimed invention "to implement the *in vivo* expression of antibody genes by cells which secrete said antibodies in the blood circulation of the mammal" is not supported by the disclosure. Applicants respectfully disagree. To implement the expression of the antibody, the invention propose a particular construction in which both polynucleotide sequences coding respectively for heavy and light antibody chains are put on each side of an Internal Ribosomal Entry Sequence (IRES) (*see,* specification, pg. 13, lines 1-6). By use of this particular construction both heavy and light polypeptide chains are expressed by translation of the same unique RNA message. This translation results in an

antibody molecule in which the chains are assembled by disulfide bridges, such as for naturally occurring antibodies.

The Examiner also alleges the lack of predictability concerning efficient therapeutic concentrations of secreted antibodies according to instant disclosure. Applicants respectfully disagree. The *in vivo* concentration of secreted antibodies in mammals sera, after implantation of genetically modified claimed cells is found to be in a range from 100 ng/ml to 300 ng/ml (*see,* specification, pg. 16, lines 12-14, in the presented examples). Such serum concentrations of monoclonal antibodies are sufficient for obtaining therapeutic effects in certain diseases. Moreover, higher antibody production by cells not naturally producing antibodies can be achieved by (i) changing the transcription promoter, (ii) changing the gene delivery system, or (iii) implanting a higher number of genetically modified cells. For example, recent experiments in the Applicants' laboratory using adenoviral gene transfer permitted the achievement of 100- to 1000-fold higher antibody concentrations in the bloodstream of treated mice. These concentrations are equal to or higher than those used with any of the currently used monoclinal antibodies for treating human beings.

The experiments with Tg10 mouse monoclonal antibody are not a model for human pathology, but rather a mammalian model to verify the levels of antibody expression and antibody secretion reached after implantation of claimed cells in a mammal. The nature and specificity of the particular antibody are not relevant for this purpose. However, the immunological test to determine concentrations of Tg10 antibody (developed by the Applicant) provides a powerful tool to measure the final concentration of the secreted antibodies and to measure the efficiency of intracellular antibody assembly and extracellular antibody secretion.

The Examiner alleges that implantation of genetically antibody-secreting myotubes in mice does not constitute an enabling disclosure that is extrapolatable to humans. Applicants respectfully disagree. Many laboratories are actually working with this approach. Moreover, muscular cells can be easy modified *in vivo* in different ways, such as saline injection, electroporation, or recombinant virus derived from adeno-associated virus or adenovirus.

Structural muscular organisation between mouse and humans are very similar. Moreover, in a living mammal, cells are arranged to form tissues, where they form very narrow intercellular

contacts and where the cells are separated by an extra-cellular matrix to which they become attached. Applicants have shown (for the first time) that antibodies produced by implanted muscular cells can migrate across this extra-cellular matrix to reach the blood circulation.

Amount of experimentation necessary

The reduction to practice of the claimed invention can be realised without difficulties by those skilled in the art, taking in account both the teaching of the specification and the standard methods already known related to gene transfer, such as expression and transcription vector construction, antibody gene structure or cell implantation methods.

35 USC § 102

Wright

The Examiner has rejected claims 1-4, 6-13 and 20 under 35 U.S.C. § 102(b) as allegedly anticipated by Wright *et al.*, 12(3,4) Crit. Rev. Immunol. 125-168 (1992) ("*Wright*"). Applicants respectfully traverse.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. MPEP § 2131. "There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *Scripps Clinic & Research Foundation v. Genentech Inc.*, 18 USPQ 2d 1001, 1010 (Fed. Cir. 1991). Here, Wright does not disclose the claimed invention.

Wright does not anticipate the pending claims. *Wright* concerns the production of monoclonal antibodies *in vitro* by non-B cell lines, with the purpose of purifying the monoclonal antibodies from the culture supernatants. *Wright* does not disclose a method for the production of monoclonal antibodies *in vivo*.

The Applicant's approach, where monoclonal antibodies are produced by genetically modified non-B cells and secreted into a mammal, is not mentioned by *Wright*. Consequently, the claims are novel over *Wright*.

Stevenson

The Examiner has rejected claims 1-4, 12, 13, 15, 16, and 20 as allegedly anticipated by Stevenson *et al.*, 772 Ann. N.Y. Acad. Sci. 212-226 (1995) ("*Stevenson*"). Applicants respectfully traverse.

Stevenson does not anticipate the pending claims. Unlike the use of the claimed cells of the invention, *Stevenson* is a straight "gene therapy" approach, which uses the injection of polynucleotide vector into the cells of the mammal, rather than the implantation of cells. *Stevenson* is a vaccine approach.

The *Stevenson* cells are forced to express artificial antibody fragments (scFv) *in vivo* by combining both variable antibody regions from heavy and from light chains by means of a binding peptide, with the intention to induce an antibody response against these scFv fragments. The antibody fragments lack constant domains, which are responsible for effector antibody functions.

Even then, *Stevenson* uses adjuvant molecules to improve the immune response against scFv. *Stevenson* does not teach that these artificial scFv fragments diffuse across interstitial fluids, nor that the scFv fragments reach the blood circulation.

By contrast, the claimed non-plasmocyte cells can produce and secrete either entire antibody molecules or antibody fragments that always comprise constant domains (to conserve antibody properties due to its effector function). Consequently, the claims are novel over *Stevenson*.

Moritz

The Examiner has rejected claims 1-10, 12, 13, 15, 16, and 20 as allegedly anticipated by Moritz *et al.*, 91 Proc. Natl. Acad. Sci. USA 4318-4322 (1994) ("*Moritz*"). The Examiner alleges that *Moritz* disclose cells not naturally producing antibodies (*e.g.* cytotoxic T-lymphocytes) containing a viral vector (retroviral) capable of expressing an antibody in the blood circulation of mammals. Applicants traverse.

Moritz does not anticipate the pending claims. *Moritz* does not introduce the recombinant cells into a mammal. Also, cytotoxic T-lymphocytes are not "non-plasmocyte cells" as recited in the claims.

Moritz takes into account a known, particular T-cell population. T-infiltrated lymphocytes (TIL) are able to infiltrate tumoral tissues. *Moritz* genetically modified TIL cells, to force them to express chimeric proteins on in the cell surface. *Moritz* combined a scFv fragment and a transmembrane protein signal transducting fragment. *Moritz* looks for a cell surface

expression of a protein of interest. The aim was to change the identification properties of the TIL cell, so as to specifically kill tumor cells.

Moritz does not disclose a secreting antibody cell, nor does *Moritz* disclose a delivery method for antibodies reach the blood circulation.

Chen

The Examiner has rejected claims 1-4, 6-12, 14-16, and 20 as allegedly anticipated by Chen *et al.*, 91 Proc. Natl. Acad. Sci. USA. 5932-5936 (1994) ("*Chen*"). The Examiner alleges that *Chen* discloses cells known not to naturally produce antibodies (COS-1 and CD4+ T lymphocytes) which are genetically modified with recombinant antibody gene-containing expression vectors for *ex vivo* gene transfer of said cells or *in vivo* gene transfer of said vectors for secretion of the recombinant antibodies into the blood circulation of mammals (*see, Chen,* Abstract and pg. 5934). Applicants traverse.

Chen does not anticipate the pending claims. *Chen* does not introduce the recombinant cells into a mammal. *Chen* relates to Fab production induced in T-cells. The aim of the *Chen* work is the production Fab fragment locally in lymphoid organs where T lymphocytes stay and where the development of HIV is made. T-lymphocytes are known by their short *in vivo* length of life term.

As discussed above concerning *Stevenson*, the disclosed antibody fragments lack the constant regions comprising the amino-acid residues responsible for effector antibody functions. Moreover, all experiences disclosed in *Chen* are made *in vitro*. No *in vivo* experiments are shown. No mention is made about a possible secretion and diffusion of the antibody fragment to reach the blood circulation.

By contrast, the genetically modified non-plasmocyte cells of the invention present a much longer length-life term.

Schlom

United States patent 5,892,019 ("*Schlom*") discloses a method for making synthetic immunoglobulin-like molecules in which (1) the variable regions from heavy and light chains are bond between them by a binding peptide, and (2) they are bound to the constant region of the heavy immunoglobulin chain in order to conserve their effector functions. This approach has

been designed by the *Schlom* because of the (then) lack of alternative methods. In fact, *Schlom* affirms (col. 1; lines 48-55) that "It is all the more inefficient to develop a transfectant synthesizing a functional antibody molecule encoded by two separate genes. Currently is it not feasible to carry out ex vivo introduction of two immunoglobulin genes simultaneously in a significant percentage of a cell population for reintroduction of the transfected cells into the host for genetic immunotherapy."

By contrast, what *Schlom* considers unfeasible is precisely the object of the instant disclosure. Applicants clearly demonstrate the feasibility of this innovative approach. The synthesis of a functional antibody molecule by an efficient transcription of two sequences coding for two separate genes is accomplished by the use of a recombinant virus expressing both light and heavy immunoglobulin chains from the same transcriptional unit by means of an IRES sequence. Applicants are able to transduce a substantial fraction of cells and to induce the whole cell population to assemble and secrete the antibody.

Schinstine

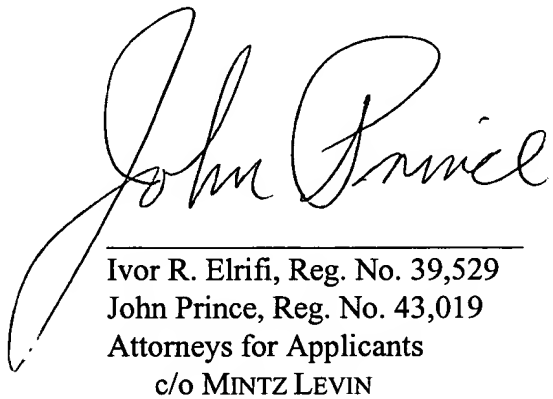
United States patent 5,853,717 ("*Schinstine*") discloses encapsulated cells in which cell proliferation is maintained under control in order to avoid the cellular death, so as to conserve as long as possible the live cells in culture. Antibody production is not mentioned. The pore size of the *Schinstine* membranes are probably not large enough to allow the antibody to cross.

This document is not related with the object of the present invention.

CONCLUSION

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact either of the undersigned at the telephone number provided below.

Respectfully submitted,



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Enclosures:

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Gene Therapy of Human Severe Combined Immunodeficiency (SCID)–X1 Disease

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Severe combined immunodeficiency–X1 (SCID-X1) is an X-linked inherited disorder characterized by an early block in T and natural killer (NK) lymphocyte differentiation. This block is caused by mutations of the gene encoding the γc cytokine receptor subunit of interleukin-2, -4, -7, -9, and -15 receptors, which participates in the delivery of growth, survival, and differentiation signals to early lymphoid progenitors. After preclinical studies, a gene therapy trial for SCID-X1 was initiated, based on the use of complementary DNA containing a defective γc Moloney retrovirus–derived vector and ex vivo infection of $CD34^+$ cells. After a 10-month follow-up period, γc transgene–expressing T and NK cells were detected in two patients. T, B, and NK cell counts and function, including antigen-specific responses, were comparable to those of age-matched controls. Thus, gene therapy was able to provide full correction of disease phenotype and, hence, clinical benefit.

In considering diseases that might be ameliorated by gene therapy, a setting in which a selective advantage is conferred by transgene expression, in association with long-lived transduced cells such as T lymphocytes, may prove critical. SCID-X1 offers a reliable model for gene therapy because it is a lethal condition that is, in many cases, curable by allogeneic bone marrow transplantation (*1–4*). It is caused by γc cytokine receptor deficiency that leads to an early block in T and NK lymphocyte differentiation (*1–3*). In vitro experiments of γc gene transfer have shown that γc expression can be restored (*5–7*), as well as T and NK cell development (*8–9*), while the immunodeficiency of γc^- mice can be corrected by ex vivo γc gene transfer into hematopoietic precursor cells (*10, 11*). Long-term expression of human γc has also been achieved by retroviral infection of canine bone marrow (*12*). It has been anticipated that γc gene transfer should confer a selective advantage to transduced lymphoid progenitor cells because, upon interaction with interleukin-7 (IL-7) and IL-15, the γc cytokine receptor subunit transmits survival and proliferative signals to T and NK lymphocyte progenitors, respectively (*2, 3*). This hypothesis received further support from the observation that a spontaneously occurring γc gene reverse mutation in a T cell precursor in one patient led to a partial, but sustained, correction of the T cell deficiency, including at least 1000 distinct T cell clones (*13, 14*). Spontaneous correction of the immunodeficiency has otherwise not been observed in several hundred γc-deficient SCID patients nor in γc^- mice (*2–4*).

Two patients, aged 11 months (P1) and 8 months (P2), with SCID-X1 met the eligibility criteria for an ex vivo γc gene therapy trial. SCID-X1 diagnosis was based on blood lymphocyte phenotype determination and findings of γc gene mutations resulting either in a tail-less receptor expressed at the membrane (P1) (R289 X) or in a protein truncated from the transmembrane domain that was not expressed at cell surface (P2) (a frameshift causing deletion of exon 6) (*15*). After marrow harvesting and $CD34^+$ cell separation, 9.8×10^6 and 4.8×10^6 $CD34^+$ cells per kilogram of body weight from P1 and P2, respectively, were preactivated, then infected daily for 3 days with the MFG γc vector–containing supernatant (*16*). $CD34^+$ cells (19×10^6 and 17×10^6/kg, respectively) were infused without prior chemoablation into P1 and P2, ~20 to 40% and 36% of which expressed the γc transgene as shown by either semiquantitative PCR analysis (P1) or immunofluorescence (P2). As early as day +15 after infusion, cells carrying the γc transgene were detectable by PCR analysis (*17*) among peripheral blood mononuclear cells. The fraction of positive peripheral blood mononuclear cells increased with time (Fig. 1). T lymphocyte counts increased from day +30 in P1 (who had a low number of autologous T cells before therapy), whereas γc-expressing T cells became detectable in the blood of P2 at day +60 (Fig. 2). Subsequently, T cell counts, including $CD4^+$ and $CD8^+$ subsets, increased to 1700/μl from day +120 to +150 and reached values of ~2800/μl after 8 months (Fig. 2). Transgenic γc protein expression could not be studied on P1 cells given the presence of the endogenous tail-less protein. However, semiquantitative PCR performed at day +150 showed that a high proportion of T cells carry and express the γc transgene (Fig. 1, A and B). Similar results were observed at day +275. Southern blot analysis of provirus integration in peripheral T cells from both patients revealed a smear indicating that multiple T cell precursors had been infected by the retroviral vector (*18*).

Immunofluorescence studies showed that γc was expressed on the membrane of T cells in P2. The magnitude of expression was similar to that of control cells (Fig. 3A), as found in previous in vitro gene transfer experiments (*5, 8, 9*). These results indicate that sufficient transgene expression had been achieved and that γc membrane expression is likely to be regulated by the availability of the other cytokine receptor subunits with which γc associates (*3*). Both αβ and γδ T

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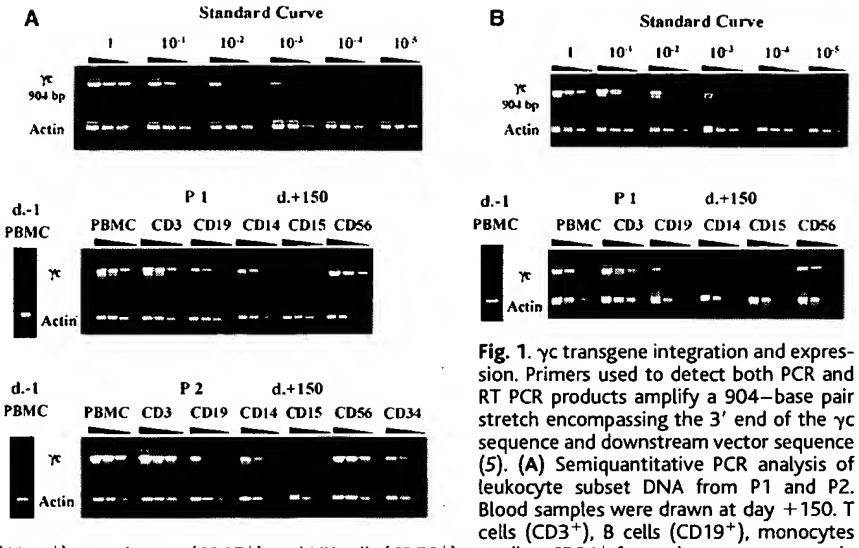


Fig. 1. γc transgene integration and expression. Primers used to detect both PCR and RT PCR products amplify a 904–base pair stretch encompassing the 3′ end of the γc sequence and downstream vector sequence (*5*). (**A**) Semiquantitative PCR analysis of leukocyte subset DNA from P1 and P2. Blood samples were drawn at day +150. T cells ($CD3^+$), B cells ($CD19^+$), monocytes ($CD14^+$), granulocytes ($CD15^+$), and NK cells ($CD56^+$) as well as $CD34^+$ from a bone marrow sample obtained at day +150 from P2 were isolated by a FACStar plus cell sorter (Becton Dickinson) after staining with appropriate mAbs (*19*). Purity was >99%. Sorted cells were analyzed for the frequency of vector-containing cells (*17*). Actin DNA was amplified in parallel. Samples from peripheral blood mononuclear cells (PBMC) obtained before treatment are shown as negative controls. A standard curve was constructed by diluting cells containing one copy of the MFG γc vector (*5*) with noninfected cells. All specimens were tested at three dilutions: 1:1, 1:20, and 1:200. (**B**) Semiquantitative RT-PCR analysis of leukocyte-subset RNA from P1. The same blood sample as in (A) was used. Actin cDNA was amplified in parallel as a control of RNA content. The standard curve was constructed as in (A) (*17*). No signal was detected in the absence of reverse transcriptase (not shown). Each specimen was diluted to 1:1, 1:500, and 1:5000.

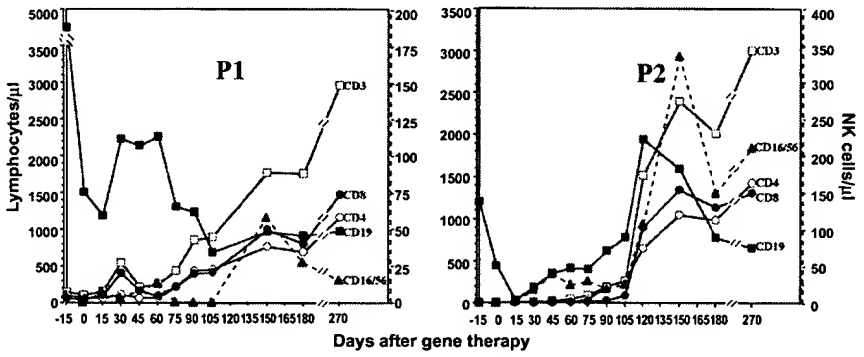


Fig. 2. Longitudinal study of lymphocyte subsets from patient 1 (P1) and patient 2 (P2). Absolute counts of T cells ($CD3^+$, $CD8^+$, and $CD4^+$), B cells ($CD19^+$), and NK cells ($CD16^+$, $CD56^+$) are shown as a function of time. Day 0 is the date of treatment. The scale for NK cells is on the right-hand side of each panel.

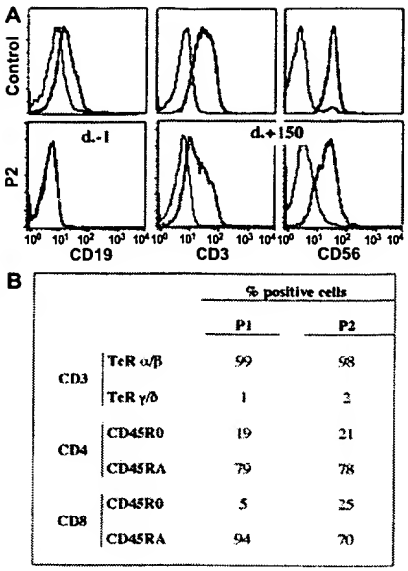
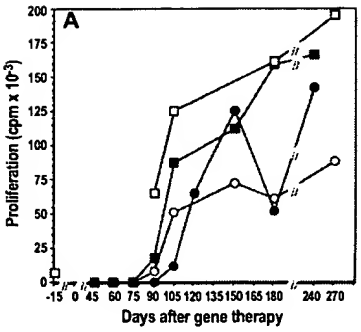


Fig. 3. γc protein expression and lymphocyte subsets. (**A**) γc protein detection at the surface of lymphocyte subsets from a control and from P2 obtained at day +150. γc expression on B cells from P2 after treatment was undetectable (not shown). The *y* axis depicts the relative cell number, and the *x* axis shows the logarithm of arbitrary immunofluorescence units. Thin lines are isotype controls; thick lines, staining by the anti-γc. Similar results were observed on blood samples obtained at days 275 (P1) and 240 (P2). (**B**) The percentage of $CD45RO^+$ and $CD45RA^+$ among CD4 and CD8 T cells from P1 and P2 obtained at day +275 and 240, respectively, as well as the percentage of T cells expressing either an αβ TCR or a γδ TCR.

cell receptor (TCR)–expressing T cells were detected (Fig. 3B). Polyclonality and V_β TCR diversity were demonstrated by using antibodies specific for TCR V_β (*19*) and the immunoscope method (*18*, *20*). In both patients, naïve $CD45RA^+$ T cells were detected, accounting for a majority of the T cell subset (Fig. 3B). In both patients, T cells proliferated from day +105 in the presence of phytohemagglutinin (PHA) and antibodies to CD3 (anti-CD3). The extent of proliferation was the same as that of age-matched controls (Fig. 4A). After primary vaccination, in vitro T cell proliferative responses to tetanus toxoid (P1 and P2: 18,000 and 12,000 cpm, respectively) and polioviruses (P2: 38,000 cpm) were observed within normal range (*21*). P1 T cells were also found to proliferate in the presence of protein pure derivitive (PPD) (12,000 cpm) as a likely consequence of bacillus Calmette-Guerin (BCG) persistence after immunization at 2 months of age in this immunocompromised child. Five months after cessation of intravenous immunoglobulin (Ig) therapy, antibodies to tetanus and diphtheria toxoids as well as to polioviruses were found in the serum of both patients, together with detectable concentrations of IgG and IgM (Fig. 4B). A normal level of IgA was also detected in the serum of P1. As determined by semi-quantitative PCR and reverse transcriptase–PCR analysis, it was observed that in both cases, a low fraction of B cells carry and express the γc transgene (Fig. 1). It is therefore unknown whether antibody responses are provided by untransduced or the few transduced B cells. Residual persistence (< 1%) of administered intravenous immunoglobulins (last given 5 months before measurement of antibody response) could, in part, also contribute. The γc-expressing NK cells were detected in the blood of P2 by day 30 (Figs. 1, 2, and 3A). These cells efficiently killed K562 cells in vitro (*18*). NK cells became detectable in the blood of P1 only from day +150.

As a likely consequence of development and sustained function of the immune system, clinical improvement was observed in both patients. In P2, protracted diarrhea as well as extensive graft-versus-host disease (GVHD)–like skin lesions disappeared. Both patients left protective isolation at days 90 and 95 and are now at home 11 and 10 months, respectively, after gene transfer without any treatment. Both enjoy normal growth and psychomotor development. No side effects have been noted. A similar result has since been achieved in a third patient 4 months



B

	IgG	IgA	IgM	Tet. tox. (iu/ml)	Dipht. Tox. (iu/ml)	Polio virus Titer x 10^{-1} I	II	III
	mg/dl			Antibodies to :				
P1 (d. + 270)	323	41	202	.53	.86	320	160	160
P2 (d. + 240)	309	0	46	.93	.63	640	640	160
Control (age matched)	420-850	16-80	40-90	>.20	>.20	>80		

Fig. 4. Functional characteristics of transduced lymphocyte subsets. (**A**) Longitudinal follow-up of PHA (□, ■)– and anti-CD3 (○, ●)–induced proliferation of lymphocytes from P1 (open symbols) and P2 (filled symbols) (*8*). Background [^{3}H]thymidine uptake was less than 400 cpm. Positive control values are >50 × 10^3 cpm. (**B**) Serum immunoglobulin analysis was determined by nephelometry and serum antibody by enzyme-linked immunosorbent assay after immunization (see above). Diphtheria toxoid (Dipht. tox.) was also used for immunization. The last intravenous Ig injections were given at day +90 in both patients. Tet. Tox., tetanus toxoid. Isohemagglutinins to blood group A have now been detected in both patients' sera.

after gene transfer (*22*). These results demonstrate that in these patients, a selective advantage was conferred to T and NK lymphocyte progenitors, enabling full-blown development of mature and functioning T and NK lymphocytes (*23*).

These overall positive results contrast with the failure of previous attempts to perform ex vivo gene therapy in adenosine deaminase (ADA)–deficient patients (*24–27*). Concomitant administration of ADA enzyme to these patients is likely to have counterbalanced the potential growth advantage of the transduced cells in this setting (*23*). Also, advances in the methodology of gene transfer into CD34$^+$ cells, i.e., the use of a fibronectin fragment (*28*) as well as of a cytokine combination enabling potent CD34 cell proliferation, contributed to the success of γc gene therapy.

Because γc gene transfer was achieved without any additional myeloablative or immunosuppressive therapy, these results pave the way for a possible extension of this therapeutic approach to other genetic diseases characterized by defective cell-subset generation, such as other forms of SCID (*29*). The kinetics of T cell development in γc gene transfer is similar to that observed in SCID patient recipients of haploidentical stem cell transplantation (*4*), suggesting that early progenitor cells have been infected by the MFG γc virus and effectively transduced. The hypothesis that transduced autologous T cells in P1 account for the development of the T cell compartment is unlikely because (i) the infected CD34$^+$ cell population was contaminated by less than 0.1% CD3$^+$ T cells; (ii) a thymic gland (27 mm by 25 mm by 25 mm at day +275) became detectable by ultrasound echography, indicative of thymopoiesis, whereas most T cells at day +275 exhibit a naïve CD45RA$^+$ phenotype; and (iii) the T cell repertoire was polyclonal and diverse. In both patients, it was shown that at day +150, a fraction of bone marrow CD34$^+$ cells harbored and expressed the γc transgene (Fig. 1, P2). It was not possible to determine whether more primitive cells, i.e., CD34$^+$CD38$^-$ cells, were transduced because of insufficient bone marrow sample. In the mouse, a common lymphoid progenitor (CLP) gives rise to the different lymphocyte populations (*30*). If a human counterpart of CLP exists, it would be the best candidate from among the earliest cells that were transduced ex vivo from these patients. Identification of integration sites in the various cell lineages could help determine the permissive differentiation stage. The question of the persistence of T and NK cell generation has yet to be addressed. If infected cells have no self-renewal capacity and have a short life-span, new generation of T and NK cells should cease. However, the fact that a thymic gland is still detectable 9 months after γc gene transfer suggests that thymopoiesis is still ongoing. Follow-up of the SCID-X1 patient in whom a spontaneous reversion mutation occurred in a T cell precursor (*13, 14*) indicates that gene transfer could be sufficient to provide a functional memory T cell pool for a number of years. This optimistic view will require careful sequential appraisal. Kohn *et al.* have previously shown that transgenes placed under the control of the long-terminal repeat (LTR) viral promoter can be silenced in quiescent T cells (*31*). Although the identification of silencing sequences in the MFG LTR makes this a strong possibility (*31*), down-regulation of γc expression has not been observed so far in these two patients, in γc-deficient mice treated by ex vivo γc gene transfer (*11*), or in cell lines maintained in culture over 1 year (*5*).

Follow-up will be required to assess the long-term effects of ex vivo γc gene transfer in CD34$^+$ cells of SCID-X1 patients. To date, this methodology has resulted in the sustained correction (up to 10 months) of the SCID-X1 phenotype in two patients, including a patient in whom the mutated protein is expressed at the cell surface. It is presumed that the effect results from a strong positive selective pressure provided to the corrected lymphoid progenitors.

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15. Patient 1 had pneumocystis carinii pneumonitis and had received BCG immunization. Patient 2 suffered from recurrent oral candidiasis, pneumocystis carinii infection, protracted diarrhea, failure to thrive, and GVHD-like skin lesions. Neither patient had an HLA (human leukocyte antigen)–identical sibling. Patients were placed in a sterile isolation ward and received nonabsorbable oral antibiotics and intravenous Igs every 3 weeks for 3 months. Parents gave informed consent for participation in the trial.
16. The defective MFG γc vector has been described previously (*5*). It was packaged in the ψcrip cell line. The MFG γc vector–containing supernatant was manufactured and provided by Genopoietic (Lyon, France) under GMP guidelines. The vector supernatant was free of replication-competent retrovirus as determined by S+L- assay and a β-galactosidase mobilization test [R. H. Bassin, N. Tuttle, P. J. Fischinger, *J. Cancer* **6**, 95 (1970); M. Printz *et al.*, *Gene Ther.* **2**, 143 (1995)]. Concentration of the virus in the supernatant was 5 × 10^5 infectious virus particles (*5*). Marrow CD34$^+$ cells were positively selected by an immunomagnetic procedure (CliniMACS, Miltenyi Biotec, Bergish Gladbach, Germany). CD34 cells were cultured in gas-permeable stem cell culture (PL-2417) containers (Nexell Therapeutics, Irvine, CA), at a concentration of 0.5 × 10^6 cells/ml in X-vivo 10 medium (Biowhittaker, Walkerville, MD) containing 4% fetal cell serum (Stem Cell Technologies, Vancouver, Canada), stem cell factor (300 ng/ml, Amgen), polyethylene glycol–megabaryocyte differentiation factor (100 ng/ml, Amgen), IL-3 (60 ng/ml, Novartis), and Flt3-L (300 ng/ml, R&D Systems, Minneapolis, MN) for 24 hours at 37°C in 5% CO_2. Containers were precoated with the CH296 human fragment of fibronectin (50 μg/ml) (TaKaRa, Shiga, Japan). Retroviral containing supernatant was added every day for 3 days. Cells were then harvested, washed twice, and infused back into the patients.
17. For semiquantitative PCR and RT-PCR analysis, DNA was isolated from the indicated cell populations. A reference standard curve was constructed by diluting cells from a SCID-X1–derived Epstein-Barr virus (EBV)–B cell line containing one copy per cell of the MFG γc provirus (*5*) in uninfected cells from the same EBV-B cell line (100, 10, 1, 0.1, 0.01, and 0.001%). DNA from each sample was also quantified by actin gel amplification. MFG γc primers sequences and

actin primers sequences are available on request. DNA was amplified in a 50 μl of PCR reaction mixture by using 30 cycles at an annealing temperature of 60°, for γc primers and 68°C for actin primers. A sample of the amplified product was separated on a 1% agarose gel and analyzed by ethidium bromide staining. RNA was prepared with the RNA easy kit (Qiagen) and was reverse-transcribed with the Superscript Preamplification System (Gibco-BRL). γc proviral and β-actin cDNA amplification were performed as described above. Quantification of expression was made by comparison with RNA isolated from the same standard curve of diluted cells.

18. M. Cavazzana-Calvo *et al.*, data not shown.
19. The following monoclonal antibodies (mAbs) were used in immunofluorescence studies: anti-γc chain: Tugh 4 (rat IgG2, PharMingen, San Diego, CA); anti-CD3: Leu 4 (IgG2a, Becton Dickinson, San Diego, CA); anti-CD4: Leu3a (IgG1, Becton Dickinson); anti-CD8: Leu 2a (IgG1, Becton Dickinson); anti-CD19: J4 119 (IgG1 Immunotech, Marseille, France); anti-CD14: Leu M3 (Becton Dickinson); anti-CD16: 3G8 (IgG1, Immunotech); anti-CD56: MY31 (IgG1, Becton Dickinson); anti-CD15 (IgM, PharMingen); anti-TCR αβ: BMA031 (IgG1, Immunotech); anti-TCR γδ: IMMU 515 (IgG1, Immunotech); anti-CD45RO: UCHL1 (IgG2a, Immunotech); anti-CD45RA: 2H4 (IgG1, Coulter Clone, Margency, France); anti-CD34: HPCA-2 (IgG1, Becton Dickinson); anti-TcR Vβ2: MPB2D5 (IgG1, Immunotech); anti-TcR Vβ3: CH92 (IgM, Immunotech); anti-TcR Vβ5.1: IMMU 157 (IgG2a, Immunotech); anti-TcR Vβ5.2: 36213 (IgG1, Immunotech); anti-TcR Vβ5.3: 3D11 (IgG1, Immunotech); anti-TcR Vβ8: 56C5.2 (IgG2a, Immunotech); anti-TcR Vβ9: FIN9 (IgG2a, Immunotech); anti-TcR Vβ13.1: IMMU 222 (IgG2, Immunotech); anti-TcR Vβ13.6: JU74.3 (IgG1, Immunotech); anti-TcR Vβ14: CAS1.13 (IgG1, Immunotech); anti-TcR Vβ17: E17.5F3.15.13 (IgG1, Immunotech); anti-TcR Vβ21.3: IG125 (IgG2, Immunotech). Fluorescence staining was done with phycoerythrin- or fluorescein isothiocyanate–conjugated mAbs. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson).
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21. Unstimulated lymphocyte proliferations were <1000 cpm. Control positive values of antigen-stimulated proliferations were >10,000 cpm.
22. This patient was treated at 1 month of age. Within 3 months, T and NK lymphocyte counts reached age-matched control values. The γc expression at T and NK cell surfaces was fully restored. The child is at home without any therapy, 4 months after treatment.
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REPORTS

Localization of a Short-Term Mem ry in *Drosophila*

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Memories are thought to be due to lasting synaptic modifications in the brain. The search for memory traces has relied predominantly on determining regions that are necessary for the process. However, a more informative approach is to define the smallest sufficient set of brain structures. The *rutabaga* adenylyl cyclase, an enzyme that is ubiquitously expressed in the *Drosophila* brain and that mediates synaptic plasticity, is needed exclusively in the Kenyon cells of the mushroom bodies for a component of olfactory short-term memory. This demonstrates that synaptic plasticity in a small brain region can be sufficient for memory formation.

The localization of memory traces has occupied neuroscientists throughout this century (*1*). Approaches have ranged from surgical ablation to mapping localized necessary gene expression in transgenic animals (*2*, *3*). Until recently, attempts to localize a memory trace have relied mainly on determining necessary brain regions (*4*). However, in a highly integrated network, other components besides the one being studied may also be necessary.

In insects, much attention has been paid to the mushroom bodies as the site for olfactory learning (*3*, *5*–*8*). In *Drosophila*, they are made up of about 2500 intrinsic neurons (Kenyon cells), receive multimodal sensory input, preferentially from the antennal lobe to the calyx, and send axon projections to the anterior brain where they bifurcate to form the α/β, α′/β′, and γ lobes (*9*). Noninvasive intervention techniques can provide mushroom body–less flies. In most respects, these flies show remarkably normal behavior but are deficient in olfactory learning (*5*). Genes important for olfactory memory have elevated expression levels in the mushroom bodies (*6*, *8*). Additionally, the mushroom bodies are necessary for context generalization in visual learning at the flight simulator and the control of spontaneous walking activity (*10*, *11*).

The *rutabaga* (*rut*) gene of *Drosophila* encodes a type I Ca^{2+}/calmodulin-dependent adenylyl cyclase (AC). Regulated synthesis of cyclic adenosine 3′,5′-monophosphate by

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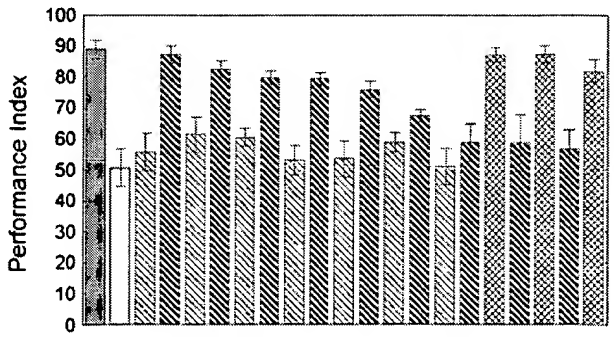

Fig. 1. The *rut* mutant defect in olfactory short-term memory can be rescued with a rut^+ cDNA in several GAL4 enhancer trap lines. Memory was measured about 2 min after classical conditioning (*17*). Performance indices (PIs) of *rut* mutant flies (white bar) and *rut* mutant flies with either a P[UAS_{GAL4}-rut^+] or GAL4 enhancer trap element (thin diagonal striped bars) were significantly different from wild-type flies (dark gray bar; P's < 0.0005). There was no significant difference between *rut* mutant flies' PIs rescued with GAL4 enhancer trap elements 247, c772, 30y, 238y, and H24 and the P[UAS_{GAL4}-rut^+] compared with wild-type flies (dark gray and thick diagonal striped bars, respectively; P's > 0.05). Mutant *rut* flies' performance was rescued with GAL4 element 201y and the P[UAS_{GAL4}-rut^+] (P < 0.05) but was also significantly lower than the performance of wild-type flies (P < 0.005). GAL4 enhancer trap lines c232, 189y, and 17d with a P[UAS_{GAL4}-rut^+] did not rescue the *rut* mutation (P's > 0.05). Wild-type flies heterozygous for GAL4 enhancer trap elements c232, 189y, and 17d were not significantly different from wild-type flies (dark gray and cross-hatched bars; P > 0.05). Bars represent mean PIs; errors are SEMs; n = 6 for all genotypes.

GENE THERAPY:

The Best of Times, the Worst of Times

W. French Anderson*

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According to recent press coverage, gene therapy has fallen on hard times. If one were to believe the news media, gene therapy is both a scientific failure and unsafe. Is this gloomy picture true? Fortunately, no. The paper by Cavazzana-Calvo *et al.* (1) on page 669 of this issue provides an example of the exciting results that are starting to be obtained in human gene therapy clinical trials. The authors have successfully treated with gene therapy (for at least up to 10 months) two infants suffering from inherited severe combined immunodeficiency (SCID).

Mutations in several different genes of immune cells can result in SCID. The first gene therapy trial almost 10 years ago treated two girls suffering from a type of SCID caused by a deficiency in the enzyme adenosine deaminase (ADA). In the new study, Cavazzana-Calvo *et al.* treat patients with an X-linked form of SCID (SCID-X1) caused by a mutation in the gene encoding the γc subunit, a component of certain cytokine receptors. After several years of preclinical studies, these investigators have carried out a clinical trial with two SCID-X1 patients, ages 11 and 8 months. They took hematopoietic stem cells (which expressed the surface marker CD34 and were capable of differentiating into all types of blood cells) from the infants' bone marrow and incubated the cells ex vivo with a retroviral vector carrying the γc cDNA. The transduced stem cells were then transfused back into the SCID-X1 patients. The authors present data from 10 months of follow-up and the results are very encouraging. Ten months after receiving transduced stem cells, the numbers of T, B, and natural killer (NK) cells of the immune system were normal, as were a number of measures of immune function (such as specific responses to antigen). Clinically, the two patients improved considerably and were able to leave protective isolation in the hospital after 3 months and have been at home ever since. Clearly, longer follow-up is necessary and more patients need to be treated, but the initial data strongly suggest that SCID-X1 can be successfully treated by retroviral-mediated gene therapy.

Why are the results of Cavazzana-Calvo *et al.* more encouraging than those from the earlier gene therapy experiments that treated ADA-deficient SCID patients (2-6)? In the first clinical protocol, the investigators inserted a normal copy of the gene encoding ADA (carried in a retroviral vector) into mature T lymphocytes (2). Later protocols attempted to transfer the same gene into bone marrow stem cells (3-5), which would differentiate into T lymphocytes capable of responding to new antigens. Cavazzana-Calvo and co-workers used a Moloney-derived retroviral vector (MFG) to deliver the therapeutic gene to the SCID-X1 infants. MFG is an improvement over the earliest retroviral vectors

and may be more effective for expressing genes in T cells, but the vector itself could not be the major reason for success; MFG has been used in a number of trials without significant efficacy. Certainly, the transduction conditions of the new study are far superior to those of the early 1990s. Of most significance, perhaps, is the inclusion of Flt3 (a factor that greatly enhances stem cell growth in culture) along with other growth factors in the medium for culturing bone marrow stem cells, and the use of fibronectin-coated culture vessels. The levels of gene transduction obtained by Cavazzana-Calvo *et al.* are, consequently, much higher than those obtained in earlier studies.

Unlike the earlier gene therapy trials that treated ADA-deficient SCID patients, Cavazzana-Calvo and colleagues did not have to administer PEG-ADA--a polyethylene glycol-conjugated ADA enzyme preparation that reduces the levels of the toxic molecule deoxyadenosine in ADA-deficient patients--to the SCID-X1 infants. The concomitant administration of PEG-ADA is believed to lessen the potential growth advantage of ADA gene-corrected cells (7). Finally, SCID-X1 can result in a more profound deficiency in T cells than ADA-deficient SCID; therefore, the positive selection for gene-corrected T cells may have been more vigorous in the SCID-X1 patients.

The majority of ADA-deficient SCID patients treated with gene-corrected stem cells have not been significantly helped. But the very first ADA-deficient SCID patient, a 4-year-old girl who received only gene-corrected mature T cells, and not stem cells, has thrived (2, 8, 9) (see the figure). She received 11 infusions from September 1990 to August 1992 and has maintained a circulating level of 20 to 25% gene-corrected T cells and a normal life-style with amelioration of her disease symptoms. Her partial response to PEG-ADA treatment before gene therapy had not provided her with an adequate immune system; nonetheless, we have felt it wise to continue treating her with PEG-ADA as a safety-net.

Ashanti de Silva. Now 13, Ashanti was the first patient to be treated with gene therapy. She received infusions of T cells that had been transduced with a gene for ADA (an enzyme that she lacks), resulting in an amelioration of the symptoms of her severe combined immunodeficiency.

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Careful monitoring indicates that the vigor of her immune response has gradually diminished over the past several years, but it still remains in the normal range, albeit at the low end. But, as she only received mature gene-corrected T cells that, unlike stem cells, cannot be educated to respond to new antigens, how has she been able to generate an immune response to the new antigens that she encounters constantly? One explanation is provided by the data obtained in the first 6 months of treatment [see figure 1A of (2)]. When only a small number of gene-corrected T cells were infused (less than 1% of the total T cell population), the total number of T cells tripled from 500 to 1500 per microliter of blood. When several infusions were missed because of technical problems, the T cell number plummeted back to 500. When infusions of gene-corrected T cells were resumed, the total T cell number again rose rapidly, this time to over 2000. One could speculate that it requires only a threshold level of "normal" (that is, gene-corrected) T cells in the lymphoid tissues to provide a microenvironment that allows the noncorrected (both immature and mature) T cells to function normally. Thus, her immature T cells may be able to differentiate in lymphoid tissue, and thereby provide her with immune protection against new antigens, as long as the "normal" mature T cells are maintained above a critical level.

The successful treatment of the first gene therapy patient suggests that the positive results of Cavazzana-Calvo *et al.* may continue over the long term. The gene-corrected stem cells of the two SCID-X1 infants should continue to experience a positive selection in the patients so that even if some cells have their γc gene silenced over time, others will expand to maintain the immune status of the patients.

In addition to the success achieved with gene therapy for the treatment of SCID, recent publications suggest progress in the treatment of hemophilia (10) and in the growth of new blood vessels to treat cardiovascular disease (11). Furthermore, early data demonstrate headway in the development of gene-based vaccines for treating several chronic infectious diseases and some types of cancer.

The field of gene therapy has been criticized for promising too much and providing too little during its first 10 years of existence. But gene therapy, like every other major new technology, takes time to develop. Antibiotics, monoclonal antibodies, organ transplants, to name just a few areas of medicine, have taken many years to mature. Major new technologies in every field, such as the manned rocket to the moon, had failures and disappointments. Early hopes are always frustrated by the many incremental steps necessary to produce "success." Gene therapy will succeed with time. And it is important that it does succeed, because no other area of medicine holds as much promise for providing cures for the many devastating diseases that now ravage humankind.

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